Lupus (2016) 25, 925–933
http://lup.sagepub.com

SPECIAL ARTICLE

Clinical significance and new detection system of autoantibodies in myositis with interstitial lung disease

R Nakashima, Y Hosono and T Mimori
Department of Rheumatology and Clinical Immunology, Kyoto University, Kyoto, Japan

Anti-aminoacyl-tRNA synthetase (ARS) and anti-melanoma differentiation-associated gene 5 (*MDA5*) antibodies are closely associated with interstitial lung disease in polymyositis and dermatomyositis. Anti-ARS-positive patients develop common clinical characteristics termed anti-synthetase syndrome and share a common clinical course, in which they respond well to initial treatment with glucocorticoids but in which disease tends to recur when glucocorticoids are tapered. Anti-*MDA5* antibody is associated with rapidly progressive interstitial lung disease and poor prognosis, particularly in Asia. Therefore, intensive immunosuppressive therapy is required for anti-*MDA5*-positive patients from the early phase of the disease. New enzyme-linked immunosorbent assays to detect anti-ARS and anti-*MDA5* antibodies have recently been established and are suggested to be efficient and useful. These assays are expected to be widely applied in daily practice. *Lupus* (2016) **25,** 925–933.

Key words: Polymyositis; dermatomyositis; clinically amyopathic dermatomyositis; interstitial lung disease; anti-aminoacyl tRNA synthetase antibody; anti-synthetase syndrome; anti-*MDA5* antibody; high resolution computed tomography; ferritin; enzyme-linked immunosorbent assay

Introduction

Polymyositis and dermatomyositis (PM/DM) are systemic autoimmune disorders that involve muscle, skin and lungs. A number of autoantibodies are detected in PM/DM patient sera, some of which are specific to PM/DM (known as myositis-specific autoantibodies; MSAs) or myositis overlap syndromes (known as myositis-associated autoantibodies; MAAs). Notably, each MSA/MAA has characteristic clinical significance, being closely associated with symptoms, complications, reactivity to therapy or prognosis of PM/DM patients. Therefore, identification and measurement of MSAs/MAAs can aid the diagnosis, classification and management of PM/DM patients. The representative MSAs/MAAs are listed in Table 1.[1–18] In this article, we review the clinical significance and the new detection systems for MSAs/MAAs specifically associated with both myositis and interstitial lung disease (ILD).

Correspondence to: Tsuneyo Mimori, Graduate School of Medicine, Kyoto University, Kawaharacho 54, Shogoin, Sakyo-ku, Kyoto City, Kyoto 606-8507, Japan.
Email: mimorit@kuhp.kyoto-u.ac.jp

MSAs associated with ILD in myositis

Among MSAs/MAAs, anti-aminoacyl-tRNA synthetase (ARS) antibodies and anti-melanoma differentiation-associated gene 5 (*MDA5*) antibodies have the closest association with ILD in PM/DM. Anti-ARS antibodies are detected in 40–60% of PM/DM-ILD cases[19–21] and anti-*MDA5* in about 25–100% of DM-ILD cases.[19,21,22] In our department, 85% of PM/DM-ILD cases were positive for anti-ARS or anti-*MDA5* (56% and 29%, respectively). With regard to DM-ILD (including clinically amyopathic DM-ILD), anti-ARS or anti-*MDA5* antibodies are detected in about 90% of patients (37% and 52%, respectively). Conversely, 79% of PM-ILD were positive for anti-ARS but anti-*MDA5* was not detected (Figure 1).

Anti-ARS antibodies

ARSs are enzymes that catalyze the binding of amino acids to the corresponding transfer RNAs to form aminoacyl-tRNAs in an energy-dependent manner. Among the 20 synthetases that correspond to the 20 amino acids, eight different ARSs have

10.1177/0961203316651748

Table 1 Myositis-specific autoantibodies and myositis-associated autoantibodies.

Autoantibodies	*Frequency*	*Significance*
Myositis-specific autoantibodies		
Anti-aminoacyl-tRNA (ARS)	~30%	Anti-synthetase syndrome: myositis, ILD, arthritis, Raynaud's phenomenon, fever, mechanic's hand
Anti-Jo-1	15–20%	
Anti-PL-7	<5%	
Anti-PL-12	<5%	
Anti-OJ	<5%	
Anti-EJ	<5%	
Anti-KS	<5%	
Anti-phenylalanyl-tRNA synthetase (Zo)	<1%	
Anti-tyrosyl-tRNA synthetase	<1%	
Anti-SRP[1–3]	5%	Severe disease, resistant to treatment, recurrent
Anti-Mi-2[4]	5–10%	Childhood and adult DM
Anti-*MDA5* (CADM-140)	20–35% of DM	CADM, rapidly progressive ILD
Anti-TIF1-γ[5–7]	20% of DM	DM, malignancy associated DM
Anti-NXP2 (MJ)[8–11]	3–15%	DM, JDM, malignancy, skin calcification in children
Anti-HMGCR[12–14]	5–8%	Necrotizing myopathy, statin-induced myopathy
Anti- SAE[15–17]	2–8% of DM	DM
Myositis-associated autoantibodies		
Anti-Ro52 (single reactivity to Ro52, not Ro60)[18]	35%	Co-occurrence with anti-Jo-1, other CTDs (especially SSc)
Anti-Ku	20–30%	Overlap myositis
Anti-U1RNP	10%	MCTD, overlap with SLE or SSc
Anti-PM-Scl	8–10%	Overlap myositis (Caucasian)

ARS: aminoacyl tRNA synthetase; ILD: interstitial lung disease; DM: dermatomyositis; CADM: clinically amyopathic dermatomyositis; JDM: juvenile dermatomyositis; SRP: signal recognition particle; HMGCR: 3-hydroxy-3-methylglutaryl-coenzyme A reductase; SAE: small ubiquitin-like modifier activating enzyme; SSc: systemic sclerosis; MCTD: mixed connective tissue disease; CTD: connective tissue disease; SLE: systemic lupus erythematosus; SSc: systemic sclerosis.

been recognized as autoantigens of MSAs, including anti-Jo-1 (histidyl-tRNA synthetase),[23,24] anti-PL-7 (threonyl),[25] anti-PL-12 (alanyl),[26] anti-EJ (glycyl),[27] anti-OJ (isoleucyl),[28] anti-KS (asparaginyl),[29] anti-tyrosyl-tRNA synthetase antibodies[30] and anti-phenylalanyl-tRNA synthetase antibodies.[31] With a few exceptions, each patient has only one of these autoantibody types; however, patients show similar clinical manifestations, characterized by myositis, ILD, arthritis, fever, Raynaud's phenomenon and mechanic's hand, which is called 'anti-synthetase syndrome (ASS)'.[32] It is noteworthy that ILD is the most prevalent extra-muscular manifestation and is found in 70–95% of anti-ARS-positive patients.[33–35] ILD of anti-ARS-positive patients tends to be diagnosed at the same time or even before the development of myositis.[32] That is, if anti-ARSs are detected in idiopathic interstitial pneumonia (IIP) patients, some may eventually develop myositis.[20] Indeed, anti-ARS antibodies can be detected in about 5–10% of unselected IIP patients.[35,36]

High resolution computed tomography (HRCT) findings of anti-ARS-positive patients with ILD show predominant involvement of the lower lung fields and the peripheral and/or the peribronchovascular region. Ground-glass opacity (GGO), intralobular reticular opacity, traction bronchiectasis and lower lobe volume loss are frequently observed.[20,37] These HRCT findings are compatible with a non-specific interstitial pneumonia pattern, whereas the usual interstitial pneumonia pattern showing honeycombing is rare.[20,35] These characteristics of anti-ARS-positive ILD are common regardless of the presence of PM/DM.[38]

Glucocorticoids are the empirical first-line therapy because both myositis and ILD in anti-ARS-positive patients respond well to initial treatment with glucocorticoids. However, additional immunosuppressive agents are often necessary because both myositis and ILD recur when glucocorticoids are tapered and glucocorticoids are associated with chronic deterioration of muscle strength and pulmonary function.[20,32,38]

Although anti-ARS-positive patients show similar clinical manifestations with ASS, some detailed clinical analyses suggest some differences in clinical manifestations among patients with different anti-ARS antibodies. Hamaguchi et al. suggested that anti-PL-12, anti-OJ and anti-KS antibodies tended to be associated with ILD rather than myositis.[33] Two other studies showed that arthritis and

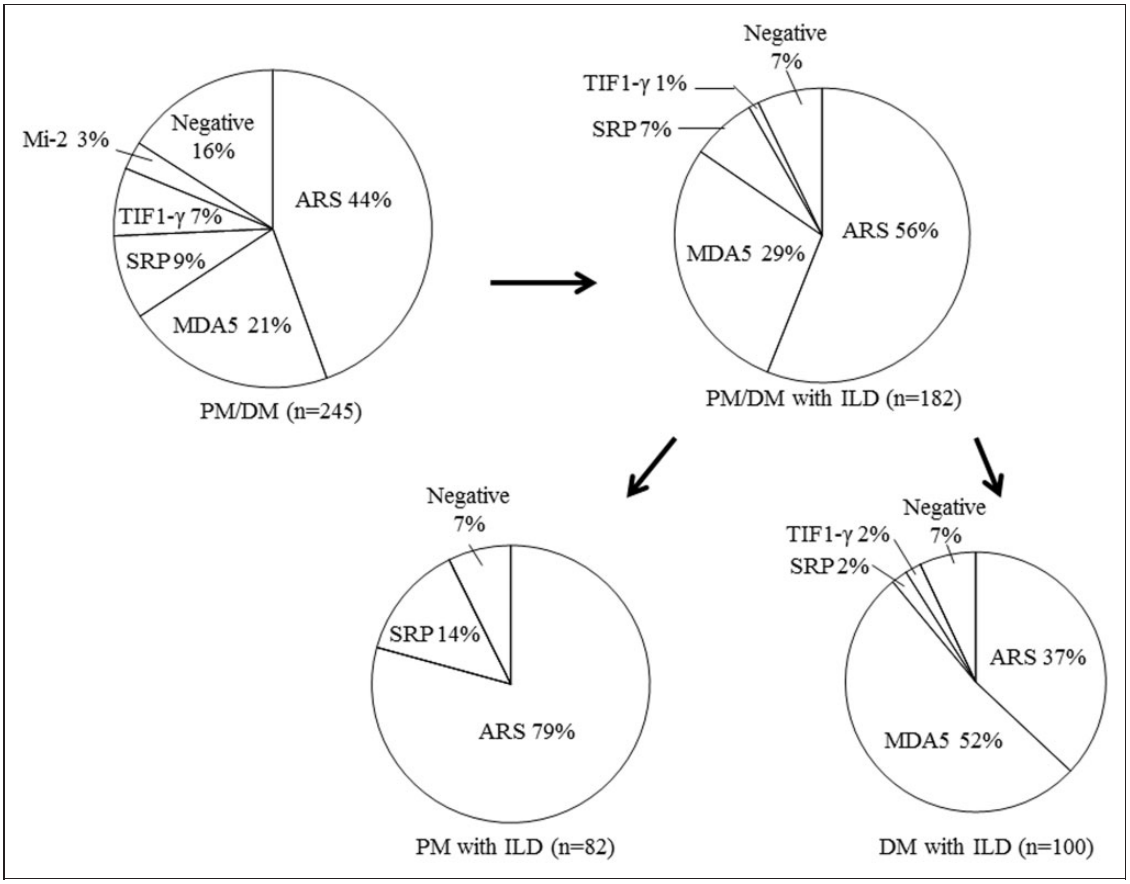

Figure 1 Profiles of myositis-specific autoantibodies (MSAs) in polymyositis and dermatomyositis (PM/DM) patients. Forty-four per cent of PM/DM patients were positive for anti-aminoacyl-tRNA synthetase (ARS) antibodies and 21% for anti-*MDA5* antibody. Among PM/DM patients with interstitial lung disease (ILD), anti-ARS or anti-*MDA5*-positive patients were more frequently detected, 56% and 29%, respectively. Anti-*MDA5* antibody was detected specifically in DM and is the most frequent MSA in DM with ILD (52%). On the other hand, anti-ARS antibody was the most frequent MSA in PM with ILD (79%).

myopathy were more frequent in anti-Jo-1 than in non-anti-Jo-1 ARS patients.[39,40] A meta-analysis also suggested that patients with non-anti-Jo-1 ARS had a greater odds ratio of presenting with fever (relative risk (RR) 0.69, 95% confidence interval (CI) 0.52–0.90) and ILD (RR 0.87, 95% CI 0.81–0.93) than those with anti-Jo-1 antibodies. The frequencies of myositis (RR 1.60, 95% CI 1.38–1.85), arthralgia (RR 1.52, 95% CI 1.32–1.76) and mechanic's hand (RR 1.47, 95% CI 1.11–1.94) were increased by almost 50% in patients with anti-Jo-1 compared to those with non-anti-Jo-1 ARS.[34] Not only clinical manifestations but also mortality appears to be different between anti-Jo-1 and non-Jo-1-anti-synthetase patients. Several reports suggested that non-anti-Jo-1 ARS patients have decreased survival compared with anti-Jo-1 patient.[39,40] However, this tendency might be explained by the fact that the diagnosis of non-anti-Jo-1-ARS patients tends to be delayed compared with anti-Jo-1 patients,[39] partly because non-anti-Jo-1 ARS antibodies are not detected because validated commercial assays have not been widely available.[39]

Anti-*MDA5* antibody

Clinically amyopathic dermatomyositis (CADM) is defined as a disorder with the typical skin manifestations of DM (heliotrope rash or Gottron's papules) but little or no evidence of clinical myositis. It was reported that CADM patients in Asia frequently develop life-threatening, rapidly progressive ILD.[41,42] In 2005, Sato et al. reported a new autoantibody named anti-CADM-140, which was specific for CADM.[43] Subsequently, the target autoantigen of anti-CADM-140 antibody was identified as *MDA5*, also known as interferon-induced with helicase C domain protein 1 (IFIH1).[44,45] Anti-*MDA5* is a DM-specific autoantibody that is

detected in 20–50% of Asian adult DM patients (including CADM) and is associated with relatively lower creatine kinase (CK) levels, a high frequency of ILD (90–95%), especially rapidly progressive ILD (RP-ILD) (50–80%), and a poor prognosis due to respiratory failure.[43,45–48] Interestingly, these characteristics of anti-*MDA5*-positive patients appear not to be the same in American and European cohorts.[49–52] The frequency of anti-*MDA5* antibodies in American and European cohorts (5–10% in adult DM) is lower than in Asian cohorts. Furthermore, anti-*MDA5* antibodies are associated with ILD (60–70%) but not with RP-ILD, in American and European cohorts.[49,50,52] Such clinical discrepancies may be explained by differences in ethnicity or environmental background. However, a recent report suggested an association between anti-*MDA5* antibodies and RP-ILD even in an American cohort[22] composed of patients with ethnicity similar to the former studies. One of the limitations of these cohort studies of anti-*MDA5* is selection bias because the patients were collected from a single centre, and so further international, multicentre investigations are still needed.

Gono et al. reported an association between anti-*MDA5* positivity and human leukocyte antigen (HLA) DRB1*0101/0405 in a Japanese cohort.[53] Muro et al. reported an increased prevalence of anti-*MDA5*-positive DM in two areas along the Kiso River in central Japan.[54] Hosono et al. suggested a seasonal occurrence from October to December was associated with the onset of anti-*MDA5*-positive DM.[55] These findings support the potential influence of genetic and environmental factors on the development of anti-*MDA5*-positive DM.

Anti-*MDA5* antibody has also been reported in juvenile dermatomyositis (JDM) cohorts and the clinical characteristics were similar to those of adult patients with anti-*MDA5*. In Japanese paediatric cohorts, anti-*MDA5* was identified in 40–60% of JDM and was associated with ILD, RP-ILD and a poor prognosis.[56,57] Conversely, in a European cohort, anti-*MDA5* was found in 7% of JDM and was associated with skin and mouth ulceration, arthritis and milder muscle disease but was not associated with RP-ILD similar to American adults.[58]

Anti-*MDA5*-positive patients have distinctive features not only in physical manifestations but also in blood tests and radiological findings. Hepatobiliary enzymes and serum ferritin levels were elevated more often in anti-*MDA5*-positive DM patients than in anti-*MDA5*-negative DM patients and serum ferritin levels correlated with the activity of ILD in anti-*MDA5*-positive patients.[54,59] Moreover, levels of serum cytokines such as interleukin (IL)-6, IL-18 and macrophage colony-stimulating factor are also elevated in anti-*MDA5*-positive patients.[60,61] These serological characteristics may indicate the activation of monocytes and macrophages in the pathophysiology of anti-*MDA5*-positive DM with intractable ILD, although other cytokines, such as IL-8, IL-10 and interferon-α are also elevated, suggesting complex mechanisms in the pathophysiology of the disease.[61–63] HRCT findings of anti-*MDA5*-positive patients were reported by Tanizawa et al. as an absence of intralobular reticular opacities and a predominance of consolidation and GGO in lower lung fields or random GGO patterns.[37]

Because of the very poor prognosis of DM patients who develop RP-ILD, combined immunosuppressive therapy has been recommended.[64,65] As anti-*MDA5* antibody is strongly associated with RP-ILD and a poor prognosis, we have recently treated anti-*MDA5*-positive patients with an intensive immunosuppressive regimen including high-dose glucocorticoids, oral calcineurin inhibitors and intravenous cyclophosphamide pulse (Figure 2) from the early stage of the disease. This approach achieved an improved survival rate of anti-*MDA5*-positive patients (Figure 3).[66] However, about 25% of anti-*MDA5*-positive patients did not survive even with this intensive therapy. Therefore, further investigation and establishment of other therapeutic strategies for these DM patients with intractable ILD is still required.

MDA5 is a cytoplasmic retinoic acid-inducible gene-I (*RIG-I*)-like receptor involved in the recognition of viral RNA leading to the expression of type 1 interferon and inflammatory cytokines.[67] The finding that *MDA5* is an autoantigen recognized by a DM-specific autoantibody is interesting because past reports have suggested a potential association between myositis and viral infections, particularly the picornaviruses Coxsackie B virus.[68–70] Recently, it was suggested that the innate immune response contributes to the pathogenesis of idiopathic inflammatory myopathy, as represented by the Jo-1-induced[71] or the C protein-induced myositis model.[72] Thus, further investigation is required to focus on the relationship between *MDA5* and the pathogenesis of DM with intractable ILD to increase our understanding of the pathophysiology of the disease and to develop more effective therapies.

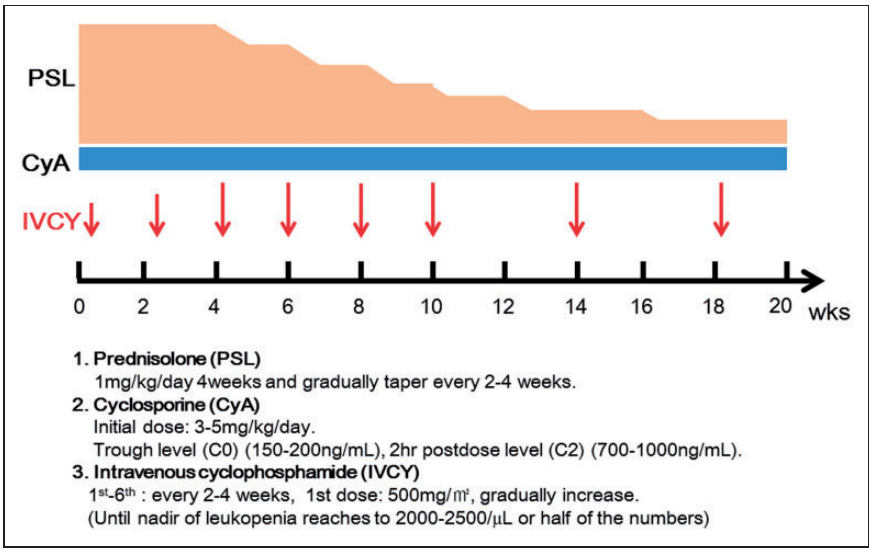

Figure 2 Intensive immunosuppresive regimen for anti-*MDA5*-positive patients with dermatomyositis (DM)-interstitial lung disease (ILD).
High dose glucocorticoids (GCs), oral cyclosporine (CyA) and intravenous cyclophosphamide pulse (IVCY) are used in combinations. Modified from Nakashima et al.[61]

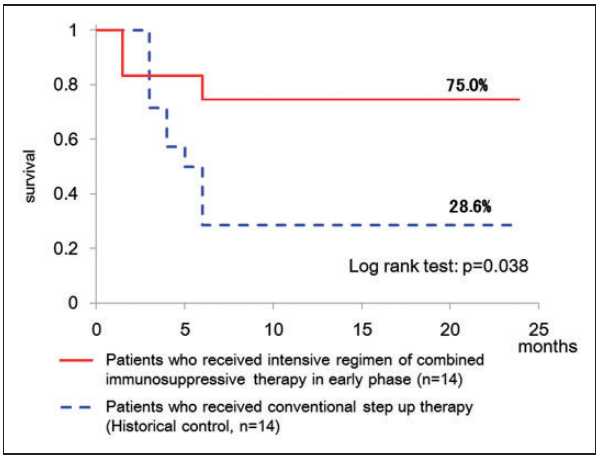

Figure 3 Survival prognosis of anti-*MDA5*-positive patients with and without an intensive immunosuppressive regimen.
Patients who received an intensive immunosuppressive regimen showed significantly better life prognosis than those who received conventional step-up therapy, 28.6% versus 75.0% of 6 months survival rate. Modified from Nakashima et al.[61]

New detection systems of MSAs

A new ELISA for measuring anti-ARS antibodies

Historically, the detection of anti-ARS antibodies has relied on RNA immunoprecipitation techniques. In this assay, HeLa cell or other tissue culture cell extracts are reacted with patient IgG-bound protein A sepharose beads, and RNA components in the immune complex are extracted, separated on a urea polyacrylamide gel, and visualized by silver staining.[73] This technique is safe and very useful for the screening of autoantibodies to RNA–protein complexes in autoimmune diseases. Anti-ARS antibodies can be identified by the characteristic electrophoretic patterns of transfer RNAs. However, this immunoprecipitation assay can only be performed in restricted laboratories because of its complicated procedure. Other assay systems such as ELISAs (only for anti-Jo-1) and line blots can also be used for detecting anti-ARS antibodies,[74,75] although all the antibodies of interest are not routinely detected. Thus, we aimed to develop a convenient quantitative ELISA to detect anti-ARS antibodies easily and simultaneously.[35]

We isolated cDNAs encoding six major synthetases reactive with autoantibodies (histidyl, threonyl, alanyl, glycyl, isoleucyl and asparaginyl-tRNA synthetases, which correspond to Jo-1, PL-7, PL-12, EJ, OJ and KS, respectively), and made recombinant proteins expressed by *Escherichia coli* or Hi-5 insect cells. Jo-1, PL-12, EJ and KS were confirmed by reactivity with their corresponding autoantibodies by immunoblot and ELISA, whereas recombinant PL-7 was only detected by ELISA but not by immunoblot. We confirmed that PL-7 had a good reactivity in its native form but not when it was denatured by sodium dodecyl sulfate (SDS) or urea, suggesting that a conformational PL-7 epitope is important for autoantibody reactivity.[35] In contrast, recombinant OJ showed poor antigenic activity even in baculovirus and

Hi-5 cell expression systems or in its native condition. This may be explained by the unique character of isoleucyl-tRNA synthetase, which is a component of a multi-enzyme complex containing eight kinds of class I synthetases and three non-enzymatic factors.[76,77] Some 'anti-OJ-positive sera' determined by an electrophoretic band pattern of immunoprecipitated proteins might recognize other components or the structural conformation of the complex. Therefore, because we considered it difficult to prepare OJ antigen as a single molecule, we used a mixture of the five recombinant synthetases except for the OJ antigen in a new ELISA system. Investigating the appropriate ratio and concentration of antigens, we found all five anti-ARS antibodies could be detected with the highest sensitivity and specificity in the new ELISA system. Finally, we confirmed the efficacy of the system by screening 694 serum samples from patients with various connective tissue diseases (CTDs) and IIP from a Japanese multicentre study that included 30 healthy controls. Except for one false positive sample, all ELISA results were consistent with the standard RNA immunoprecipitation (sensitivity 100% and specificity 99.8%, compared with RNA immunoprecipitation).[35] Anti-ARS antibodies were detected in 30.8% of PM/DM and 2.5% of other CTDs. Interestingly, anti-ARSs were also positive in 10.7% of IIP patients.[35]

Our data suggest that this new ELISA will be useful for the diagnosis and classification of PM/DM, differential diagnosis of myopathy and ILD, predicting prognosis and deciding the treatment of PM/DM-associated ILD. Moreover, although further investigation will be needed, it might also be useful for monitoring disease activity of PM/DM by quantification of anti-ARS titres. This ELISA system is now manufactured by Medical & Biological Laboratories Co. Ltd. (MBL, Nagoya, Japan), and after a multicentre trial, it was approved for diagnostic use by the health insurance system in Japan in 2014.

The limitation of this assay is the lack of distinguishing each type of anti-ARS antibody. Indeed, as reviewed above, there are some differences in the clinical manifestations and prognosis among patients with different ARS antibodies. However, different treatments for different anti-ARSs have not been established, and anti-ARS-positive patients are treated with the expectation that they share a common pathogenesis and clinical course. Therefore, the ELISA system is an efficient and reasonable method for the detection of anti-ARS as a first screen. A line blot assay for the multiple detection of MSAs/MAAs (EUROLINE Myositis Profile 3, EUROIMMUN, Luebeck, Germany) has been used, in which anti-Jo-1, PL-7, PL-12, EJ and OJ are included as separate analytes. This system can detect and discriminate MSAs/MAAs simultaneously, but it does not include anti-KS, which has a stronger association with IIP than myositis.

Another limitation is our inability to detect anti-OJ antibody by the ELISA, but this may not significantly affect the sensitivity of the assay because the prevalence of anti-OJ is the lowest among the six ARS antibodies.[78] The efficiency of this newly established ELISA system was satisfactory because the sensitivity and specificity of the system compared well with the RNA immunoprecipitation (93.5% and 99.8%, respectively), even if anti-OJ-positive sera were not excluded.[35]

An ELISA system for measuring anti-MDA5

MSAs that recognize non-RNA-binding proteins can be screened by a protein immunoprecipitation assay using ^{35}S-methionine-labelled cells as an antigen source. Using this method, anti-*MDA5* antibodies are identified by immunoprecipitating the 140 kDa protein. However, this assay is difficult to perform routinely because of the use of radioisotopes and it is technically cumbersome for diagnostic laboratories.

Sato et al. established an ELISA system for efficiently detecting anti-*MDA5* antibodies,[44] which used purified, recombinant full-length MDA5 protein expressed in COS-7 cells coated on ELISA plates. The ELISA system had a sensitivity of 85% and specificity of 100% compared with protein immunoprecipitation assay. The authors reported that the ELISA system was useful in diagnosing CADM (sensitivity of 69% and specificity of 99.6%) and predicting RP-ILD (sensitivity of 82% and specificity of 97%).[44]

Interestingly, anti-*MDA5* titres before treatment tended to be higher in patients with anti-*MDA5*-positive DM with RP-ILD who did not respond to the treatment and died than they were in the patients who responded to therapy and survived. Moreover, the titre of anti-*MDA5* antibody was significantly decreased to below the cutoff level after treatment in those who responded to the treatment.[79] For this reason, the ELISA for anti-*MDA5* appears to be useful for both the diagnosis of DM as well as in monitoring disease activity and determining the prognosis of anti-*MDA5*-positive DM with ILD. This ELISA system for anti-*MDA5* antibody is commercially available (MBL Co. Ltd., Nagoya, Japan).

Conclusion

In this review, we have highlighted the clinical importance of anti-ARS and anti-*MDA5* antibodies in PM/DM with ILD. The newly developed ELISA detection systems are expected to be widely applied in daily practice, but further improvement of the anti-ARS detection system is needed to discriminate between the different anti-ARSs and for the detection of anti-OJ. Anti-ARS and anti-*MDA5* antibodies as well as other MSAs have characteristic clinical associations, and their usefulness for the diagnosis, classification and management of PM/DM is widely recognized. Thus, developing systems to identify other MSAs easily is also required.

Acknowledgement

A part of this article was presented in the 12th Dresden Symposium on Autoantibodies, 22–26 September 2015, in Dresden, Germany.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: The authors collaborated with MBL Co. Ltd. in establishing the new ELISA system for anti-ARS antibodies and have no conflicts of interest with MBL Co. Ltd., in terms of a financial, non-financial, professional, or personal relationship.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: A part of this study was supported by the grant-in-aid for scientific research from the Ministry of Education, Science, Sports and Culture and by the grant from the Practical Research Project for Rare/Intractable Disease in Japan Agency for Medical Research and Development (AMED).

References

1 Kao AH, Lacomis D, Lucas M, Fertig N, Oddis CV. Anti-signal recognition particle autoantibody in patients with and patients without idiopathic inflammatory myopathy. *Arthritis Rheum* 2004; 50: 209–215.
2 Reeves WH, Nigam SK, Blobel G. Human autoantibodies reactive with the signal-recognition particle. *Proc Natl Acad Sci USA* 1986; 83: 9507–9511.
3 Targoff IN, Johnson AE, Miller FW. Antibody to signal recognition particle in polymyositis. *Arthritis Rheum* 1990; 33: 1361–1370.
4 Targoff IN, Reichlin M. The association between Mi-2 antibodies and dermatomyositis. *Arthritis Rheum* 1985; 28: 796–803.
5 Fujimoto M, Hamaguchi Y, Kaji K, *et al*. Myositis-specific anti-155/140 autoantibodies target transcription intermediary factor 1 family proteins. *Arthritis Rheum* 2012; 64: 513–522.
6 Kaji K, Fujimoto M, Hasegawa M, *et al*. Identification of a novel autoantibody reactive with 155 and 140 kDa nuclear proteins in patients with dermatomyositis: an association with malignancy. *Rheumatology (Oxford)* 2007; 46: 25–28.
7 Targoff IN, Mamyrova G, Trieu EP, *et al*. A novel autoantibody to a 155-kd protein is associated with dermatomyositis. *Arthritis Rheum* 2006; 54: 3682–3689.
8 Ceribelli A, Fredi M, Taraborelli M, *et al*. Anti-MJ/NXP-2 autoantibody specificity in a cohort of adult Italian patients with polymyositis/dermatomyositis. *Arthritis Res Ther* 2012; 14: R97.
9 Espada G, Maldonado Cocco JA, Fertig N, Oddis CV. Clinical and serologic characterization of an Argentine pediatric myositis cohort: identification of a novel autoantibody (anti-MJ) to a 142-kDa protein. *J Rheumatol* 2009; 36: 2547–2551.
10 Gunawardena H, Wedderburn LR, Chinoy H, *et al*. Autoantibodies to a 140-kd protein in juvenile dermatomyositis are associated with calcinosis. *Arthritis Rheum* 2009; 60: 1807–1814.
11 Ichimura Y, Matsushita T, Hamaguchi Y, *et al*. Anti-NXP2 autoantibodies in adult patients with idiopathic inflammatory myopathies: possible association with malignancy. *Ann Rheum Dis* 2012; 71: 710–713.
12 Allenbach Y, Drouot L, Rigolet A, *et al*. Anti-HMGCR autoantibodies in European patients with autoimmune necrotizing myopathies: inconstant exposure to statin. *Medicine (Baltimore)* 2014; 93: 150–157.
13 Ge Y, Lu X, Peng Q, Shu X, Wang G. Clinical characteristics of anti-3-hydroxy-3-methylglutaryl coenzyme A reductase antibodies in Chinese patients with idiopathic inflammatory myopathies. *PLoS One* 2015; 10: e0141616.
14 Mammen AL, Chung T, Christopher-Stine L, *et al*. Autoantibodies against 3-hydroxy-3-methylglutaryl-coenzyme A reductase in patients with statin-associated autoimmune myopathy. *Arthritis Rheum* 2011; 63: 713–721.
15 Betteridge ZE, Gunawardena H, Chinoy H, *et al*. Clinical and human leucocyte antigen class II haplotype associations of autoantibodies to small ubiquitin-like modifier enzyme, a dermatomyositis-specific autoantigen target, in UK Caucasian adult-onset myositis. *Ann Rheum Dis* 2009; 68: 1621–1625.
16 Muro Y, Sugiura K, Akiyama M. Low prevalence of anti-small ubiquitin-like modifier activating enzyme antibodies in dermatomyositis patients. *Autoimmunity* 2013; 46: 279–284.
17 Tarricone E, Ghirardello A, Rampudda M, Bassi N, Punzi L, Doria A. Anti-SAE antibodies in autoimmune myositis: identification by unlabelled protein immunoprecipitation in an Italian patient cohort. *J Immunol Methods* 2012; 384: 128–134.
18 Schulte-Pelkum J, Fritzler M, Mahler M. Latest update on the Ro/SS-A autoantibody system. *Autoimmun Rev* 2009; 8: 632–637.
19 Ikeda N, Takahashi K, Yamaguchi Y, Inasaka M, Kuwana M, Ikezawa Z. Analysis of dermatomyositis-specific autoantibodies and clinical characteristics in Japanese patients. *J Dermatol* 2011; 38: 973–979.
20 Hozumi H, Enomoto N, Kono M, *et al*. Prognostic significance of anti-aminoacyl-tRNA synthetase antibodies in polymyositis/dermatomyositis-associated interstitial lung disease: a retrospective case control study. *PLoS One* 2015; 10: e0120313.
21 Gono T, Kaneko H, Kawaguchi Y, *et al*. Cytokine profiles in polymyositis and dermatomyositis complicated by rapidly progressive or chronic interstitial lung disease. *Rheumatology (Oxford)* 2014; 53: 2196–2203.
22 Moghadam-Kia S, Oddis CV, Sato S, Kuwana M, Aggarwal R. Anti-*MDA5* is associated with rapidly progressive lung disease and

poor survival in US patients with amyopathic and myopathic dermatomyositis. *Arthritis Care Res (Hoboken)* 2016; 68: 689–694.
23 Nishikai M, Reichlin M. Heterogeneity of precipitating antibodies in polymyositis and dermatomyositis. Characterization of the Jo-1 antibody system. *Arthritis Rheum* 1980; 23: 881–888.
24 Mathews MB, Bernstein RM. Myositis autoantibody inhibits histidyl-tRNA synthetase: a model for autoimmunity. *Nature* 1983; 304: 177–179.
25 Mathews MB, Reichlin M, Hughes GR, Bernstein RM. Anti-threonyl-tRNA synthetase, a second myositis-related autoantibody. *J Exp Med* 1984; 160: 420–434.
26 Bunn CC, Bernstein RM, Mathews MB. Autoantibodies against alanyl-tRNA synthetase and tRNAAla coexist and are associated with myositis. *J Exp Med* 1986; 163: 1281–1291.
27 Targoff IN, Trieu EP, Plotz PH, Miller FW. Antibodies to glycyl-transfer RNA synthetase in patients with myositis and interstitial lung disease. *Arthritis Rheum* 1992; 35: 821–830.
28 Targoff IN, Trieu EP, Miller FW. Reaction of anti-OJ autoantibodies with components of the multi-enzyme complex of aminoacyl-tRNA synthetases in addition to isoleucyl-tRNA synthetase. *J Clin Invest* 1993; 91: 2556–2564.
29 Hirakata M, Suwa A, Nagai S, *et al*. Anti-KS: identification of autoantibodies to asparaginyl-transfer RNA synthetase associated with interstitial lung disease. *J Immunol* 1999; 162: 2315–2320.
30 Betteridge Z, Gunawardena H, North J, Slinn J, McHugh N. Anti-synthetase syndrome: a new autoantibody to phenylalanyl transfer RNA synthetase (anti-Zo) associated with polymyositis and interstitial pneumonia. *Rheumatology (Oxford)* 2007; 46: 1005–1008.
31 Hashish LTE, Sadanandan P, Targoff IN. Identification of autoantibodies to tyrosyl-tRNA synthetase in dermatomyositis with features consistent with antisynthetase syndrome [abstract]. *Arthritis Rheum* 2005; 52: S312.
32 Yoshifuji H, Fujii T, Kobayashi S, *et al*. Anti-aminoacyl-tRNA synthetase antibodies in clinical course prediction of interstitial lung disease complicated with idiopathic inflammatory myopathies. *Autoimmunity* 2006; 39: 233–241.
33 Hamaguchi Y, Fujimoto M, Matsushita T, *et al*. Common and distinct clinical features in adult patients with anti-aminoacyl-tRNA synthetase antibodies: heterogeneity within the syndrome. *PLoS One* 2013; 8: e60442.
34 Lega JC, Fabien N, Reynaud Q, *et al*. The clinical phenotype associated with myositis-specific and associated autoantibodies: a meta-analysis revisiting the so-called antisynthetase syndrome. *Autoimmun Rev* 2014; 13: 883–891.
35 Nakashima R, Imura Y, Hosono Y, *et al*. The multicenter study of a new assay for simultaneous detection of multiple anti-aminoacyl-tRNA synthetases in myositis and interstitial pneumonia. *PLoS One* 2014; 9: e85062.
36 Watanabe K, Handa T, Tanizawa K, *et al*. Detection of antisynthetase syndrome in patients with idiopathic interstitial pneumonias. *Respir Med* 2011; 105: 1238–1247.
37 Tanizawa K, Handa T, Nakashima R, *et al*. HRCT features of interstitial lung disease in dermatomyositis with anti-CADM-140 antibody. *Respir Med* 2011; 105: 1380–1387.
38 Takato H, Waseda Y, Watanabe S, *et al*. Pulmonary manifestations of anti-ARS antibody positive interstitial pneumonia – with or without PM/DM. *Respir Med* 2013; 107: 128–133.
39 Aggarwal R, Cassidy E, Fertig N, *et al*. Patients with non-Jo-1 anti-tRNA-synthetase autoantibodies have worse survival than Jo-1 positive patients. *Ann Rheum Dis* 2014; 73: 227–232.
40 Hervier B, Devilliers H, Stanciu R, *et al*. Hierarchical cluster and survival analyses of antisynthetase syndrome: phenotype and outcome are correlated with anti-tRNA synthetase antibody specificity. *Autoimmun Rev* 2012; 12: 210–217.
41 Ye S, Chen XX, Lu XY, *et al*. Adult clinically amyopathic dermatomyositis with rapid progressive interstitial lung disease: a retrospective cohort study. *Clin Rheumatol* 2007; 26: 1647–1654.
42 Suda T, Fujisawa T, Enomoto N, *et al*. Interstitial lung diseases associated with amyopathic dermatomyositis. *Eur Respir J* 2006; 28: 1005–1012.
43 Sato S, Hirakata M, Kuwana M, *et al*. Autoantibodies to a 140-kd polypeptide, CADM-140, in Japanese patients with clinically amyopathic dermatomyositis. *Arthritis Rheum* 2005; 52: 1571–1576.
44 Sato S, Hoshino K, Satoh T, Fujita T, Kawakami Y, Kuwana M. RNA helicase encoded by melanoma differentiation-associated gene 5 is a major autoantigen in patients with clinically amyopathic dermatomyositis: association with rapidly progressive interstitial lung disease. *Arthritis Rheum* 2009; 60: 2193–2200.
45 Nakashima R, Imura Y, Kobayashi S, *et al*. The RIG-I-like receptor IFIH1/*MDA5* is a dermatomyositis-specific autoantigen identified by the anti-CADM-140 antibody. *Rheumatology (Oxford)* 2010; 49: 433–440.
46 Hoshino K, Muro Y, Sugiura K, Tomita Y, Nakashima R, Mimori T. Anti-*MDA5* and anti-TIF1-gamma antibodies have clinical significance for patients with dermatomyositis. *Rheumatology (Oxford)* 2010; 49: 1726–1733.
47 Chen F, Wang D, Shu X, Nakashima R, Wang G. Anti-*MDA5* antibody is associated with A/SIP and decreased T cells in peripheral blood and predicts poor prognosis of ILD in Chinese patients with dermatomyositis. *Rheumatol Int* 2012; 32: 3909–3915.
48 Koga T, Fujikawa K, Horai Y, *et al*. The diagnostic utility of anti-melanoma differentiation-associated gene 5 antibody testing for predicting the prognosis of Japanese patients with DM. *Rheumatology (Oxford)* 2012; 51: 1278–1284.
49 Fiorentino D, Chung L, Zwerner J, Rosen A, Casciola-Rosen L. The mucocutaneous and systemic phenotype of dermatomyositis patients with antibodies to *MDA5* (CADM-140): a retrospective study. *J Am Acad Dermatol* 2011; 65: 25–34.
50 Ceribelli A, Fredi M, Taraborelli M, *et al*. Prevalence and clinical significance of anti-*MDA5* antibodies in European patients with polymyositis/dermatomyositis. *Clin Exp Rheumatol* 2014; 32: 891–897.
51 Labrador-Horrillo M, Martinez MA, Selva-O'Callaghan A, *et al*. Anti-*MDA5* antibodies in a large Mediterranean population of adults with dermatomyositis. *J Immunol Res* 2014; 2014: 290797.
52 Hall JC, Casciola-Rosen L, Samedy LA, *et al*. Anti-melanoma differentiation-associated protein 5-associated dermatomyositis: expanding the clinical spectrum. *Arthritis Care Res (Hoboken)* 2013; 65: 1307–1315.
53 Gono T, Kawaguchi Y, Kuwana M, *et al*. Brief report: Association of HLA-DRB1*0101/*0405 with susceptibility to anti-melanoma differentiation-associated gene 5 antibody-positive dermatomyositis in the Japanese population. *Arthritis Rheum* 2012; 64: 3736–3740.
54 Muro Y, Sugiura K, Hoshino K, Akiyama M, Tamakoshi K. Epidemiologic study of clinically amyopathic dermatomyositis and anti-melanoma differentiation-associated gene 5 antibodies in central Japan. *Arthritis Res Ther* 2011; 13: R214.
55 Hosono Y, Nakashima R, Imura Y, *et al*. The onsets of myositis with myositis-specific autoantibodies (MSAs) are associated with the seasons. *Ann Rheum Dis* 2013; 72(Suppl 3): 656.
56 Kobayashi I, Okura Y, Yamada M, Kawamura N, Kuwana M, Ariga T. Anti-melanoma differentiation-associated gene 5 antibody is a diagnostic and predictive marker for interstitial lung diseases associated with juvenile dermatomyositis. *J Pediatr* 2011; 158: 675–677.
57 Ikeda S, Arita M, Morita M, *et al*. Interstitial lung disease in clinically amyopathic dermatomyositis with and without anti-*MDA-5* antibody: to lump or split? *BMC Pulm Med* 2015; 15: 159.
58 Tansley SL, Betteridge ZE, Gunawardena H, *et al*. Anti-*MDA5* autoantibodies in juvenile dermatomyositis identify a distinct clinical phenotype: a prospective cohort study. *Arthritis Res Ther* 2014; 16: R138.
59 Gono T, Kawaguchi Y, Ozeki E, *et al*. Serum ferritin correlates with activity of anti-*MDA5* antibody-associated acute interstitial lung disease as a complication of dermatomyositis. *Mod Rheumatol* 2011; 21: 223–227.
60 Gono T, Sato S, Kawaguchi Y, *et al*. Anti-*MDA5* antibody, ferritin and IL-18 are useful for the evaluation of response to treatment in interstitial lung disease with anti-*MDA5* antibody-positive dermatomyositis. *Rheumatology (Oxford)* 2012; 51: 1563–1570.
61 Nakashima R, Mimori T. Anti-*MDA5* (melanoma differentiation-associated gene 5) antibody and dermatomyositis with rapidly

progressive interstitial pneumonia. *Nihon Rinsho Meneki Gakkai Kaishi* 2013; 36: 71–76.
62 Gono T, Kaneko H, Kawaguchi Y, *et al*. Cytokine profiles in polymyositis and dermatomyositis complicated by rapidly progressive or chronic interstitial lung disease. *Rheumatology (Oxford)* 2014; 53: 2196–2203.
63 Horai Y, Koga T, Fujikawa K, *et al*. Serum interferon-alpha is a useful biomarker in patients with anti-melanoma differentiation-associated gene 5 (*MDA5*) antibody-positive dermatomyositis. *Mod Rheumatol* 2015; 25: 85–89.
64 Kameda H, Nagasawa H, Ogawa H, *et al*. Combination therapy with corticosteroids, cyclosporin A, and intravenous pulse cyclophosphamide for acute/subacute interstitial pneumonia in patients with dermatomyositis. *J Rheumatol* 2005; 32: 1719–1726.
65 Tanaka F, Origuchi T, Migita K, *et al*. Successful combined therapy of cyclophosphamide and cyclosporine for acute exacerbated interstitial pneumonia associated with dermatomyositis. *Intern Med* 2000; 39: 428–430.
66 Nakashima R, Hosono Y, Yukawa N, *et al*. Clinical features and treatment of dermatomyositis patients with anti-CADM-140 (melanoma differentiation-associated protein 5: MDA5) antibody: recommendation of combined immunosuppressive therapy with intensive intravenous cyclophosphamide. *Arthritis Rheum* 2011; 63: S225.
67 Kato H, Takeuchi O, Sato S, *et al*. Differential roles of *MDA5* and *RIG-I* helicases in the recognition of RNA viruses. *Nature* 2006; 441: 101–105.
68 Bowles NE, Dubowitz V, Sewry CA, Archard LC. Dermatomyositis, polymyositis, and Coxsackie-B-virus infection. *Lancet* 1987; 1: 1004–1007.
69 Chevrel G, Calvet A, Belin V, Miossec P. Dermatomyositis associated with the presence of parvovirus B19 DNA in muscle. *Rheumatology (Oxford)* 2000; 39: 1037–1039.
70 Christensen ML, Pachman LM, Schneiderman R, Patel DC, Friedman JM. Prevalence of Coxsackie B virus antibodies in patients with juvenile dermatomyositis. *Arthritis Rheum* 1986; 29: 1365–1370.
71 Ascherman DP. Role of Jo-1 in the immunopathogenesis of the anti-synthetase syndrome. *Curr Rheumatol Rep* 2015; 17: 56.
72 Okiyama N, Sugihara T, Oida T, *et al*. T lymphocytes and muscle condition act like seeds and soil in a murine polymyositis model. *Arthritis Rheum* 2012; 64: 3741–3749.
73 Forman MS, Nakamura M, Mimori T, Gelpi C, Hardin JA. Detection of antibodies to small nuclear ribonucleoproteins and small cytoplasmic ribonucleoproteins using unlabeled cell extracts. *Arthritis Rheum* 1985; 28: 1356–1361.
74 Ghirardello A, Bendo R, Rampudda ME, Bassi N, Zampieri S, Doria A. Commercial blot assays in the diagnosis of systemic rheumatic diseases. *Autoimmun Rev* 2009; 8: 645–649.
75 Ghirardello A, Rampudda M, Ekholm L, *et al*. Diagnostic performance and validation of autoantibody testing in myositis by a commercial line blot assay. *Rheumatology (Oxford)* 2010; 49: 2370–2374.
76 Targoff IN. Autoantibodies to aminoacyl-transfer RNA synthetases for isoleucine and glycine. Two additional synthetases are antigenic in myositis. *J Immunol* 1990; 144: 1737–1743.
77 Quevillon S, Robinson JC, Berthonneau E, Siatecka M, Mirande M. Macromolecular assemblage of aminoacyl-tRNA synthetases: identification of protein–protein interactions and characterization of a core protein. *J Mol Biol* 1999; 285: 183–195.
78 Mahler M, Miller FW, Fritzler MJ. Idiopathic inflammatory myopathies and the anti-synthetase syndrome: a comprehensive review. *Autoimmun Rev* 2014; 13: 367–371.
79 Sato S, Kuwana M, Fujita T, Suzuki Y. Anti-CADM-140/*MDA5* autoantibody titer correlates with disease activity and predicts disease outcome in patients with dermatomyositis and rapidly progressive interstitial lung disease. *Mod Rheumatol* 2013; 23: 496–502.